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# The effects of green tea extract on teratogenicity induced by low frequency electromagnetic field on bone marrow Balb/C mice embryo

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**ABSTRACT**

**Introduction:** Electromagnetic fields produce free radicals which might be teratogen. *Camellia sinensis* is rich in natural antioxidants and antioxidants can neutralize free radicals effects. In present research the effect of *C. sinensis* extract in reduction of teratogenicity induced by electromagnetic field with 50 gauss intensity was studied on bone marrow of Balb/C mice fetuses.

**Methods:** In this experimental study, 24 Balb/C pregnant mice were randomly divided into four groups: control, sham exposed (off position), experimental 1 (electromagnetic field with 50-gauss intensity) and experimental 2 (treatment by *C. sinensis* extract + electromagnetic field with 50-gauss intensity). After treatment period, the bone marrow aspirates of Balb/C mice embryos were prepared and studied by Giemsa. The quantitative data were analyzed by Kruskal-Wallis and Kolmogorov-Smirnov using SPSS16 software at the level of $p<0.05$.

**Results:** The mean number of promyelocytes, myelocytes, erythrocytes, necrotic and apoptotic cells in experimental group1 compared with sham exposed embryos showed significant increase but the mean number of eosinophils in experimental group 1 compared with sham exposed embryos showed significant decrease. The mean number of promyelocyte and erythrocyte in experimental group 2 compared with experimental group 1 showed significant decrease. The mean of necrotic and apoptotic cells, in experimental group 2 compared with experimental group 1 showed significant increase.

**Conclusion:** Usage of *C. sinensis* can decrease the damage due to teratogenicity induced by low frequency electromagnetic field in some cells.

*Implication for health policy/practice/research/medical education:*
*Camellia sinensis* offsets the change in the number of eosinophils and promyelocytes caused by electromagnetic field and hence its consumption is recommended for radiologists and broadcasting and telecommunication personnel.

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## Introduction

Today, use of many electromagnetic waves-generating instruments is unavoidable in human's progressing industrial life. Use of the instruments such as radio, television, satellite, mobile phone, and computer has prompted many to study their biological impacts (1). The studies indicated that low-intensity electromagnetic fields contributed greatly to the prevalence of fetal developmental disorders like reduced growth of motor limbs (2), gastrointestinal and cardiovascular diseases, and disorders in hematopoietic and lymphoid tissues (3). Some studies demonstrated that one-hour radiation of 1800 MHz did not increase the levels of DNA damage in trophoblast cells, but a considerable increase was noted in the damage to the genetic material of these cells after being exposed to the radiation of different types of signals and increasing the duration of 1.8 GHz radiation for consecutive 5 minutes on the field and consecutive 10 minutes off the field and various GSM signals (4), indicating the impact of electromagnetic field intensity on

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the cells. In addition, the contribution of low-frequency electromagnetic field to chromosomal damage (5), and the increase in white and red blood cells, hemoglobin, and blood proteins (6) has been reported.

Electromagnetic waves have been shown to exert their effects on biological systems through generation or increase in reactive oxygen species (ROS). ROS, as a medium, contributes to numerous biological impacts including DNA damage and mutation induction (7). The increase in oxidative stress in hematopoietic centers has also been reported due to use of mobile phone (8). Some studies indicated that antioxidants prevented deleterious effects of these destructive agents on the body through destroying and/or decreasing free radicals. *Camellia sinensis* is a strong destroyer of free radicals (9). Also, *C. sinensis* acts as a flavonoid-rich antioxidant and inhibits postlaparotomy intra-abdominal adhesion in rats (10). A large number of useful effects of *C. sinensis* are related to epigallocatechin-3-gallate (EGCG). In the conducted investigations, the contribution of *C. sinensis* to metabolic syndrome was demonstrated (11). *C. sinensis* has inhibitory effects against free radicals and heart diseases and causes decrease in cholesterol, triglyceride, and cardiovascular diseases (12). *C. sinensis* contains large amounts of vitamins C (ascorbic acid) and E and hence exerts a stronger antioxidants property compared to these vitamins (13). Also, it has anti-allergic properties, inhibits plaque formation in the brain, decreases glucose, triglyceride, and cholesterol of the blood, inhibits lipid concentration and increases energy consumption in the body, and stimulates lipid catabolism in the liver (14). A study examined the protective effect of *C. sinensis* polyphenols on the electromagnetic radiation-induced wounds in cortical neurons of the rats exposed to 1800 MHz mobile phone for 24 hours, indicating that microwaves induced markers of cell death in these neurons and *C. sinensis* recovered the wounds. Moreover, Liu *et al*. demonstrated that *C. sinensis* inhibited the nervous damages caused by UV-C radiation which induced wounds in cerebral cortical neurons in the rats (15). Therefore, regarding the mentioned impacts of *C. sinensis*, as a strong antioxidant, the present study was done to examine the effect of *C. sinensis* extract accompanied with low-frequency electromagnetic field on bone marrow in mouse fetuses.

## Materials and Methods

This laboratory, experimental study was conducted in Animal Development Research Laboratory, Islamic Azad University of Mashhad. The Balb/C mice were purchased from Serum Institute of Mashhad, reproduced in the Animal Growth Room, and kept at 23±1 °C temperature, 65-70% humidity, and 12 light/12 dark cycle in polycarbonate cages with wire stainless lids, washed and disinfected twice a week. They were fed with a standard, pre-made meal (Javaneh Khorasan, Mashhad, Iran). To ensure the animals maturity, we used 25- to 3-month animals weighing 25-30 gr. The intercourse between male and female mice was monogamous and the day of observing vaginal plug was considered as the zero day of pregnancy. The electromagnetic field generating system with 50-Hz frequency available in Research Center for Animal Development Research Laboratory, Islamic Azad University of Mashhad (devised and made by Baharara and Ashraf) was used.

Dried *C. sinensis* was purchased from Refah Company (Lahijan, Iran). Hydroalcoholic extract of *C. sinensis* was prepared using maceration method. For extraction, the dried leaves were pulverized by an electronic mill. Then, 50 gr of the obtained powder was mixed with alcohol 80%. The mixture was left for 48 hours and stirred each 2-3 hours. After 48 hours, the mixture was filtrated using filter paper four to five times and the obtained solution, after centrifugation, was poured into rotary for solvent separation. Finally, the extract was dried in freeze dryer and kept refrigerated at 4 °C temperature (16). The dried extract was pulverized to be used more conveniently in later steps. In this research, the extract of *C. sinensis* was intraperitoneally (ip) injected at 100 ml/kg BW (17,18). To prepare the required amounts, an adequate amount of the hydroalcoholic extract of dried powder was suspended in the water. Before each injection, the mice were weighed and the injection was done at ml/kg BW.

To do the experiments, 24 pregnant female Balb/C mice were randomly assigned into 4 groups as follows:

The control group comprised the mice kept in normal conditions in Animals Growth Room.

The exposed sham group comprised the mice kept in vitro, inside the turned-off electromagnetic field-generating system within the days 9-19 of pregnancy for 4 hours (8-12 a.m.) per day. The experimental group 1 comprised the mice exposed to electromagnetic filed with 50-Hz frequency and 50-gauss intensity within the days 9-19 of pregnancy for 4 hours (8-12 a.m.) per day.

The experimental group 2 comprised the mice exposed to electromagnetic filed with 50-Hz frequency and 50-gauss intensity within the days 9-19 of pregnancy for 4 hours (8-12 a.m.) per day and intraperitoneally administered with hydroalcoholic *C. sinensis* extract at 100 ml/kg BW dose.

All pregnant mice were anesthetized on the day 19 of pregnancy using chloroform, then the fetuses were removed from the wombs, and the femurs were isolated and the bone marrow aspirates were prepared on the lam. The obtained aspirates were air-dried and the animal characteristics were labeled on them. Then, the aspirates were fixed in methanol, placed in Giemsa stain 20% (Rooz Azmoon Co., Iran) for 20 minutes, and finally additional color was washed with water. The bone marrow aspirates were counted using an optical microscope (Nikon, Japan) at 1000X magnification. The granulocyte cells such as myeloblasts, promyelocytes, myelocytes, metamyelocytes, band cells, neutrophils, eosinophils, basophils, and other hemocytoblast cells, megakaryocytes, and depleted cells were counted in the mice fetuses.

The data were analyzed by SPSS 16 software using statistical tests of Kruskal-Wallis and Kolmogorov-Simonov at 0.05 level of significance.

In all steps of proper maintenance of the animals, such as provision of their life needs, and anesthesia at dissection, ethical principles were precisely and fully observed. Each group was decided to comprise six pregnant mice.

## Results

Statistical comparison of the data of control and exposed sham samples indicated no significant difference ($p<0.05$). In addition, the treatment had no notable effect on morphology of bone marrow cells and no change was observed in their apparent form.

In statistical comparison of bone marrow cells, the mean number of promyelocytes and myelocytes in the experimental group 1 increased significantly compared to the exposed sham ($p=0.037$). In addition, the mean number of erythrocytes and depleted cells increased significantly in the experimental group 1 compared to the exposed sham ($p<0.001$), but the mean number of eosinophils in the experimental group 1 decreased significantly compared to the exposed sham ($p=0.039$), while the mean number of hemocytoblasts in the experimental group 1 increased partially compared to the exposed sham and this increase was not statistically significant ($p>0.05$; Table 1). The quantitative data of aspirates indicated that the mean number of myelocytes, erythrocytes and depleted cells in the experimental group 2 increased significantly compared to the exposed sham ($p>0.05$) while the mean number of promyelocytes, metamyelocytes, neutrophils, band neutrophils, eosinophils, and hemocytoblasts indicated no significant increase in the experimental group 2 compared to the exposed sham ($p>0.05$) and the mean number of myeloblasts, basophils, and megakaryocytes decreased in the experimental group 1 compared to the exposed sham, but the difference was not statistically significant ($p>0.05$; Table 1).

Statistical comparison of the cells indicated the mean number of promyelocytes and erythrocytes in the experimental group 2 decreased significantly compared to the experimental group 1 ($p<0.05$), but the mean number of metamyelocytes, neutrophils, band neutrophils, eosinophils, and basophils increased compared to the experimental group 1 and this difference was not statistically significant ($p>0.05$). In addition, the mean number of depleted cells increased significantly in the experimental group 2 compared to the experimental group 1 ($p=0.002$) while the mean number of myeloblasts, myelocytes, megakaryocytes, and hemocytoblasts decreased partially in the experimental group 2 compared to the experimental 1, but the difference was not statistically significant ($p>0.05$).

## Discussion

The present study aimed to evaluate the effect of *C. sinensis* extract in reduction of teratogenicity induced by electromagnetic field with low frequency on bone marrow of Balb/C mice fetuses. Comparison of the number of bone marrow cells in the fetuses exposed to electromagnetic field with that in the exposed sham indicated a significant increase in the mean number of promyelocytes, myelocytes, erythrocytes, and depleted cells and a significant decrease in the mean number of eosinophils. In this research, electromagnetic field increased cell proliferation in the majority of cells. In another study, microwave radiations on bone marrow cells in rats led to an increase in the number of metamyelocytes, band neutrophils, and mitotic cells in non-adult group and the increase in the number of metarubricytes, myelocytes, mitotic cells, and all other types of cells in the adult (19).

In addition, the increase in likelihood of DNA damage and cell proliferation was reported in brain cells of the rats exposed to electromagnetic fields (20). These findings are consistent with the present study findings on the increase in the number of most cells in the group exposed to the waves.

On the other hand, the decreased cell proliferation was demonstrated in some bone marrow cells in the present study, which is consistent with the findings of a work in which the decrease in the number of bone marrow eosinophils was reported in the group under electromagnetic field compared to the exposed sham (21). In fact, this is related to cytotoxic and genotoxic effect of electromagnetic fields. In this line, Erdal *et al*. studied the

**Table 1.** Changes in born marrow parameters in different groups

| Indexes | Groups | | | | P |
|---|---|---|---|---|---|
| | Control | Sham | Experimental 1 | Experimental 2 | |
| Myeloblasts | 54.38±9.15 | 62.07±10.43 | 64.19±7.33 | 57.25±9.76 | 0.421 |
| Promyelocytes | 21.62±3.94 | 22.06±5.70 | 29.69±4.90 | 24.00±6.80 | 0.048* |
| Neutrophilic myelocytes | 10.31±1.58 | 9.56±2.09 | 13.62±3.16 | 13.50±2.13 | 0.002** |
| Myeloblasts | 36.69±5.25 | 37.94±7.21 | 29.69±8.66 | 39.38±7.23 | 0.015* |
| Neutrophilic metamyelocyte | 67.62±13.08 | 65.94±10.22 | 57.50±11.27 | 66.75±9.13 | 0.077 |
| Neutrophilic bandcells | 117.94±24.07 | 118.06±23.45 | 103.80±21.70 | 118.75±27.77 | 0.143 |
| Polymorphonuclears | 6.88±2.30 | 6.56±1.99 | 4.19±2.17 | 6.75±2.37 | 0.001** |
| Eosinophils | 2.31±0.94 | 2.31±1.30 | 1.81±2.10 | 2.12±1.35 | 0.312 |
| Basophils | 4.56±1.09 | 4.81±0.91 | 4.75±0.88 | 5.00±1.00 | 0.862 |
| Proerythroblasts | 4.50±0.96 | 4.94±0.34 | 14.06±1.98 | 9.43±1.39 | <0.001*** |
| Hemocytoblasts | 23.31±4.82 | 22.25±4.10 | 26.62±4.16 | 23.40±4.70 | 0.021 |
| Megakaryocytes | 4.12±1.25 | 4.19±1.32 | 5.44±1.59 | 3.93±1.07 | 0.024 |
| Degenerated and apoptotic cells | 19.00±3.89 | 18.56±3.52 | 26.12±6.04 | 37.20±7.22 | <0.001*** |

The data are shown as Mean±SD; *, **, and *** significant at 0.05, 0.01, and 0.001 respectively.

effect of 0.5-mT, 50-Hz sinusoidal electromagnetic field on rat bone marrow cells and found no significant difference in the number of cells from the controls (22). But, Scarfi *et al*. found that 50-Hz field had a proliferative effect on human blood cells (23). The studies indicated that several factors were involved in causing biologic effects by electromagnetic fields, of which the most important ones were physical specs of the field, the duration of exposure, the type of tissue, and the developmental stage of the laboratory sample (24).

The conducted research on induction of cell death by electromagnetic fields in different cells indicated that these waves could cause some inducing symptoms in the cells, with final products influencing cell cycle. These products were previously recognized as stress proteins (25), but it has been generally known that these proteins are widely varied in different cells, with impacts observed as not only stoppage of cell cycle but also inconsistently as the increased rate of cell division; therefore, electromagnetic fields seem to have a dual role in cell division and the impact on different organs could vary, so that it could be represented sometimes as decrease and sometimes as increase in the number of cells.

Of other findings of the present study, the increased erythrocytes in the fetuses of the experimental group 1 compared to the exposed sham could be mentioned. This is consistent with the increased white and red blood cells in Wistar rats exposed to the stable 128 mT-intensity electromagnetic field for 5 consecutive days one hour a day (26).

Some studies associated the increase in free radicals with magnetic nanoparticles and genotoxic function of electromagnetic waves on DNA and hematopoietic stem cells (27), confirming the effect of electromagnetic fields on bone marrow as a part of hematopoietic system. Theoretically, very weak fields are not strong enough to break chemical bands of DNA molecules and hence their genetic influence could be indirectly exerted, which could together lead to the increase in freed radicals and/ or influence the enzymatic process contributing to the cell repair (22). Influencing mitochondria, apoptotic routes, heat shock proteins, metabolism of free radicals, cellular proliferation and differentiation, DNA destruction, and plasma membrane destruction are some of the mechanisms through which electromagnetic radiations exert their impact (28).

In the present study, *C. sinensis* offset the decrease in eosinophils and the increase in bone marrow promyelocytes, both caused by electromagnetic fields. Similarly, *C. sinensis* returned the change in the levels of minerals including iron, manganese, and zinc due to the impact of 900-mHz electromagnetic fields in hepatic and ovarian cells to a normal level in pigs (29). The other finding of the present study was the decrease in the depleted cell in both groups treated with the field and with both the field and the extract, which seems to be due to the increase in stress in the pregnant mice and induction of apoptosis. Since EGCG inhibits the proliferation of human hepatic cells (Hepg2) through induction of apoptosis and blocking cell cycle in the phase G1 (30,31), it could be the potential choice explaining positive effects of *C. sinensis* extract in this regard. In addition, *C. sinensis*, by the findings of most previous works, is likely to be able to contribute to the decreased electromagnetic field-induced chromosomal damages through destroying free radicals, inhibiting oxidative stress, inhibiting DNA destruction, inducing apoptosis in the damaged cells, controlling cell cycle, and the changes in genes transcription, proteins folding, heat shock proteins production, and/or specific enzymes blocking (32).

## Conclusion

The results of the present study indicated that *C. sinensis* extract offsets the change in the number of eosinophils and promyelocytes due to treatment with electromagnetic field and hence its consumption is mainly recommended for radiologists and broadcasting and telecommunication personnel. Further research can help to expand its medical and commercial applications.

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## Authors' contributions

All the authors wrote the manuscript equally.

## Conflict of interests

The authors declared no competing interests.

## Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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